RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v58.4879
Majalah Kedokteran Bandung. 2026;58(2):123–131

Majalah Kedokteran Bandung (MKB)

Received: April 1, 2026
Accepted: June 19, 2026
Available online: June 30, 2026

Impact of *CYP3A4* and *CYP3A5* Genetic Variants on Tacrolimus Dosing and Therapeutic Outcomes in Kidney Transplant Recipients

Hafiza Fareeha Bashir, Rasheeda Bashir, Farheen Aslam, Sana Rafaqat, Amina Latif, Areej Fatima, Zunaira Tahir
Department of Biotechnology, Lahore College for Women University Lahore, Pakistan

Abstract

Tacrolimus is the cornerstone of immunosuppressive therapy in kidney transplantation; however, its narrow therapeutic index and variable metabolism make dose optimization difficult. Genetic variations in the *CYP3A4* and *CYP3A5* genes significantly influence tacrolimus metabolism yet limited data are available for Pakistani population. This study aimed to evaluate the association of CYP3A4 and CYP3A5 polymorphisms with tacrolimus dose requirements and post-transplant outcomes in Pakistani kidney transplant recipients. A case-control study was conducted from January 2022 to December 2022 at Shaikh Zayed Hospital and Pakistan Kidney and Liver Institute. A total of 240 participants, including renal transplant recipients and controls, were enrolled using non-probability purposive sampling. Genotyping of *CYP3A4* and *CYP3A5* variants was performed using Tetra ARMS-PCR. Clinical data regarding tacrolimus dose requirements and rejection episodes were collected, and statistical analysis was performed using the SPSS version 25. Among the participants, 190 (79.1%) were male and 50 (20.9%) were female. Renal transplant recipients receiving tacrolimus accounted for 57% of the study population, while 43% were controls. Patients with the *CYP3A5* expressor genotype required significantly higher tacrolimus doses to achieve target trough concentrations compared to non-expressors (OR=4.060, 95% CI: 2.455–6.715, p<0.001). The CC genotype of rs10264272 was associated with increased tacrolimus dose requirements, whereas AT and TT genotypes of rs413003343 were linked to lower dose requirements and better graft outcomes. These findings suggest that CYP3A4 and CYP3A5 genotyping may support individualized tacrolimus therapy in Pakistani kidney transplant recipients.

Keywords: : CYP3A4, CYP3A5, kidney transplant, pharmacogenetics, Pakistan, tacrolimus

Introduction

According to a 2025 World Health Organization report, kidney transplantation has been performed in Pakistan since 1979, with the annual number of procedures gradually increasing from approximately 50 to 1,000 among adult and pediatric patients with end-stage renal disease.[1] Tacrolimus, a calcineurin inhibitor, is integral to kidney transplant management. However, its therapeutic effectiveness is limited by marked interindividual pharmacokinetic variability, which increases the risks of both graft rejection and drug toxicity. Even small deviations in drug exposure may have serious consequences: subtherapeutic concentrations increase the risk of rejection, whereas supratherapeutic concentrations predispose patients to nephrotoxicity, neurotoxicity, and metabolic complications.[2,3] Genetic polymorphisms in the cytochrome P450 enzyme system, particularly *CYP3A4* and CYP3A5, are major contributors to this variability because these enzymes are primarily responsible for tacrolimus metabolism and are key determinants of drug clearance and dose requirements.[4] Evidence from pediatric and adult transplant cohorts indicates that *CYP3A5* expressors require higher tacrolimus doses to achieve target trough concentrations, whereas non-expressors reach higher blood concentrations with lower doses.[5] In addition, combining *CYP3A4* variants, such as *CYP3A4* *22, with *CYP3A5* polymorphisms may improve the prediction of tacrolimus metabolism and dosing requirements.[6] In Indian kidney transplant recipients, genotype-guided dosing resulted in earlier attainment of target tacrolimus concentrations and fewer dose adjustments than conventional body-weight-based dosing.[7]

Corresponding Author:
Rasheeda Bashir
Department of Biotechnology, Lahore College for Women University Lahore, Pakistan
Email: rasheeda.bashir@lcwu.edu.pk

The CYP3A5*3/3 genotype (rs776746) causes loss of enzymatic function, resulting in higher tacrolimus concentrations and lower dose requirements, whereas *CYP3A5* expressors (1/1 or 1/3) exhibit greater clearance and require higher doses.[7,8] Similarly, the *CYP3A4* *22 variant (rs35599367) reduces enzyme expression and metabolic activity, thereby increasing tacrolimus concentrations.[7,9] The CYP3A5*1 allele is more common in populations of African and Asian ancestry and is generally associated with higher tacrolimus dose requirements. The prevalence of these genetic variants differs substantially among ethnic groups.[10] Population-based studies have demonstrated pharmacogenetic effects in Brazilian,[11] Romanian,[12] Tunisian,[13] Vietnamese,[14] and Mexican[15] populations.

Despite research on *CYP3A4* and *CYP3A5* polymorphisms in diverse populations, data from Pakistan remain scarce. To our knowledge, this is the first study to investigate *CYP3A4* and *CYP3A5* polymorphisms in Pakistani kidney transplant recipients. Given Pakistan's ethnic diversity and the potential for population-specific pharmacogenetic profiles, international dosing guidelines derived largely from Western or East Asian cohorts may not adequately address the needs of local transplant recipients. This limitation may delay attainment of therapeutic concentrations, increase the need for dose adjustments, and contribute to poorer early post-transplant outcomes. Therefore, determining the distribution of *CYP3A4* (rs35599367, rs4646437, and rs2740574) and *CYP3A5* (rs776746, rs10264272, and rs413003343) variants in Pakistani kidney transplant recipients, as well as their effects on tacrolimus dosing and clinical outcomes, is clinically important. Such information could support population-specific genotype-guided dosing strategies and improve drug safety, treatment effectiveness, and resource utilization. Incorporating pharmacogenetic data into therapeutic drug monitoring may also help optimize immunosuppressive therapy, reduce the risk of rejection, and improve long-term graft survival in resource-limited settings. This study therefore aimed to evaluate the associations of *CYP3A4* and *CYP3A5* genetic polymorphisms with tacrolimus dose requirements and post-transplant outcomes in Pakistani kidney transplant recipients.

Methods

This case-control study was conducted from January to December 2022 at Shaikh Zayed Hospital and the Pakistan Kidney and Liver Institute. It compared *CYP3A4* and *CYP3A5* genotype frequencies between kidney transplant recipients and healthy controls and evaluated the associations of these polymorphisms with tacrolimus dose requirements and post-transplant outcomes among transplant recipients. Ethical approval was obtained from the Institutional Review Board of the Department of Kidney Transplantation at Shaikh Zayed Hospital and the Department of Biotechnology at Lahore College for Women University. Written informed consent was obtained from all participants before enrollment.

Non-probability purposive sampling was used because kidney transplant recipients receiving tacrolimus represented a specialized and limited population during the study period. A total of 240 participants aged 10–50 years were enrolled, including 137 kidney transplant recipients and 103 age- and sex-matched healthy controls. The sample size was calculated using an estimated effect size of 0.3, 80% statistical power, and a significance level of 0.05, yielding a minimum required sample of 220 participants. To account for potential attrition, 240 participants were recruited.

Eligible transplant recipients had undergone living-related kidney transplantation within the preceding 12 months and were receiving tacrolimus-based immunosuppressive therapy. Patients receiving other immunosuppressive agents, such as cyclosporine or sirolimus, were excluded. Individuals who had used herbal or homeopathic medicines or nephrotoxic drugs, including ibuprofen or amikacin, within the preceding 14 days were also excluded. Healthy controls were age- and sex-matched individuals with no history of kidney disease or transplantation.

Demographic and clinical data, including age, sex, ethnicity, body weight, time since transplantation, tacrolimus dose, serum creatinine level, tacrolimus trough concentration, liver function test results, and graft rejection episodes, were obtained from medical records. Body weight was recorded to calculate weight-adjusted tacrolimus doses. Time since transplantation and concomitant medications known to inhibit or induce *CYP3A4* or *CYP3A5* activity were documented and included as covariates in the statistical analysis. Alanine aminotransferase, aspartate aminotransferase, and serum bilirubin levels were recorded to account for hepatic function as a potential

confounder of tacrolimus metabolism. In addition to tacrolimus, recipients received standard maintenance immunosuppressive therapy according to institutional transplant protocols, including mycophenolate mofetil and corticosteroids. Concomitant immunosuppressive medications were recorded for all participants and considered in the analysis because of their potential effects on graft outcomes and tacrolimus pharmacokinetics.

Tacrolimus dose requirement was defined as the mean daily dose (mg/kg/day) required to maintain a target trough concentration of 5–15 ng/mL during the first 12 months after transplantation. Therapeutic drug monitoring was performed under standardized conditions for all recipients. Tacrolimus trough concentrations were measured in blood samples collected immediately before the morning dose, approximately 12 hours after the preceding dose. All samples were analyzed using the same chemiluminescent microparticle immunoassay platform (ARCHITECT i1000SR; Abbott) to ensure consistency and comparability throughout the study. Acute graft rejection was defined according to Banff biopsy criteria. When biopsy results were unavailable, a persistent increase in serum creatinine of more than 25% above baseline, after other causes had been excluded, was considered a clinical indicator of rejection.

Under aseptic conditions, 5 mL of peripheral blood was collected in ethylenediaminetetraacetic acid Vacutainer tubes and stored at 4°C until processing. DNA was extracted using the nonorganic salting-out method described by Lahiri and Nurnberger (1991). DNA quality and concentration were assessed using a NanoDrop 2000/2000c spectrophotometer (Thermo Scientific), and samples with an A260/A280 ratio of 1.8–2.0 were considered acceptable. Three *CYP3A4* single-nucleotide polymorphisms (rs35599367, rs4646437, and rs2740574) and three *CYP3A5* polymorphisms (rs776746, rs10264272, and rs413003343) were investigated. Allele-specific primers were designed using Primer1 software, and genotyping was performed using tetra-primer amplification refractory mutation system polymerase chain reaction, followed by electrophoresis on 2% agarose gels for allele identification. Polymerase chain reaction amplification consisted of initial denaturation at 95°C for 2 minutes; 35 cycles of denaturation at 95°C for 40 seconds, annealing at single-nucleotide-polymorphism-specific temperatures for 30 seconds, and extension at 72°C for 1 minute; and a final extension at 72°C for 2 minutes.

Statistical analyses were performed using SPSS version 25. Continuous variables are presented as means and standard deviations, whereas categorical variables are presented as frequencies and percentages. Independent-samples t tests were used to compare continuous variables between groups, and chi-square tests were used to assess genotype distributions and Hardy-Weinberg equilibrium. Dominant, recessive, and codominant genetic models were analyzed. Logistic regression was used to assess associations between genetic polymorphisms and tacrolimus dose requirements or graft rejection after adjustment for age, sex, body weight, liver function parameters, time since transplantation, and concomitant medications. Multivariable logistic regression models were constructed to evaluate the independent associations of *CYP3A4* and *CYP3A5* polymorphisms with acute graft rejection after adjustment for the same covariates. Fisher's exact test was used when expected cell counts were less than five. To account for multiple comparisons, the Benjamini-Hochberg false discovery rate correction was applied. Unless otherwise stated, all reported p values are false discovery rate-adjusted values.

Results

A total of 240 participants were enrolled, including 137 (57.1%) kidney transplant recipients receiving tacrolimus-based immunosuppressive therapy and 103 (42.9%) age- and sex-matched healthy controls. Overall, 190 (79.2%) participants were male and 50 (20.8%) were female, yielding a male-to-female ratio of approximately 3.8:1. Male participants predominated in both groups. Among transplant recipients, 111 (81.0%) were male and 26 (19.0%) were female; the control group included 79 (76.7%) males and 24 (23.3%) females. The mean age was 36.9±9.1 years among transplant recipients and 34.7±7.4 years among controls, indicating comparable age distributions. All transplant recipients were Pakistani and represented several geographic regions of Punjab, including Lahore, Gujranwala, Faisalabad, and surrounding areas. Lahore residents constituted the largest proportion (45.9%), followed by participants from Gujranwala (18.9%), Faisalabad (6.8%), and other regions (31.3%). A similar regional distribution was observed among controls. The mean serum creatinine

Table 1 Demographic and Clinical Characteristics of Kidney Transplant Recipients and Controls

Parameters	Kidney Transplant Recipients n (%)	Controls n (%)
Sample Size (n)	137	103
Gender		
Male	111 (81)	79 (76)
Female	26 (19)	24 (23)
Age in years (Mean±SD)	36.9±9.1	34.7±7.4
Ethnicity		
Lahore	68 (45.9)	52 (50.5)
Gujranwala	26 (18.9)	20 (19.4)
Faisalabad	10 (6.8)	9 (8.7)
Others	33 (31.3)	22 (21.3)
Serum creatinine level (mg/dL)	0.8±0.5	0.6±0.2
Tacrolimus Dose (mg)		
1 mg	36 (24.3)	
0.5 mg	101 (68.2)	
Tacrolimus Duration (months)		
<3 months	42 (28.4)	
3–6 months	37 (25.0)	
7–9 months	47 (31.8)	
10–12 months	11 (7.4)	
Tacrolimus trough level (ng/mL)	5.30±1.4	

Data are presented as frequency (percentage) or mean±standard deviation. NA, not applicable; ng, nanogram; mg, milligram; dL, deciliter

level was 0.8±0.5 mg/dL among transplant recipients and 0.6±0.2 mg/dL among controls. Regarding tacrolimus therapy, 101 (73.7%) recipients received a maintenance dose of 0.5 mg and 36 (26.3%) received 1 mg. Acute graft rejection episodes during the 12-month follow-up were identified either by biopsy according to Banff criteria or by a persistent increase in serum creatinine of more than 25% above baseline after other causes had been excluded. Rejection events and their classifications were obtained from medical records and included in subsequent genotype-outcome analyses.

Genotype and allele frequencies of *CYP3A4* and *CYP3A5* polymorphisms in kidney transplant recipients and controls are presented in Tables 2 and 3. Genotype distributions in the control group were consistent with Hardy-Weinberg equilibrium for all investigated polymorphisms. Among the *CYP3A4* polymorphisms, rs35599367 was significantly associated with tacrolimus dose requirements and graft rejection risk. Genotype frequencies among recipients were 25.5% for GG, 42.3% for GC, and 32.1% for CC. Compared with the GG genotype, the heterozygous GC genotype was significantly associated with tacrolimus dose variability (OR=0.471, 95% CI: 0.241–0.921; p=0.028). In the allelic analysis, the C allele was more frequent among recipients and was significantly associated with a higher risk of acute graft rejection than the G allele (OR=4.060, 95% CI: 2.455–6.715; p<0.001). For rs4646437, genotype frequencies among recipients were 84.6% for CC, 13.9% for CT, and 1.5% for TT. Compared with the CC genotype, the CT genotype was significantly associated with tacrolimus dose variability (OR=0.373, 95% CI: 0.143–0.972; p=0.043). The T allele was associated with increased susceptibility to acute graft rejection (OR=2.644, 95% CI: 1.112–6.287;

Table 2 Allelic and Genotypic Frequencies of *CYP3A4* Polymorphisms in Kidney Transplant Recipients and Controls

SNP	Model	Allele/ Genotype	Kidney Transplant Recipients n (%)	Controls n (%)	OR (95% CI)	p value
rs35599367	Additive	G allele	128 (46.7)	89 (78.1)	Reference	—
		C allele	146 (53.3)	25 (21.9)	4.06 (2.46–6.72)	<0.001
	Genotypic	GG	35 (25.5)	32 (30.4)	Reference	—
		GC	58 (42.3)	25 (23.8)	0.47 (0.24–0.92)	0.028
		CC	44 (32.1)	48 (45.7)	1.19 (0.64–2.24)	0.582
	Dominant	GC+CC vs GG	102 (74.5)	73 (69.5)	0.28 (0.15–0.53)	<0.001
	Recessive	CC vs GC+GG	44 (32.1)	48 (45.7)	0.97 (0.52–1.80)	0.921
	Codominant	CC vs GC+GG	44 (32.1)	1 (0.9)	26.97 (3.62–201.14)	0.001
rs4646437	Additive	C allele	251 (91.6)	202 (96.7)	Reference	—
		T allele	23 (8.4)	7 (3.3)	2.64 (1.11–6.29)	0.027
	Genotypic	CC	116 (84.6)	98 (93.3)	Reference	—
		CT	19 (13.9)	6 (5.7)	0.37 (0.14–0.97)	0.043
		TT	2 (1.5)	1 (1.0)	0.59 (0.05–6.63)	0.670
	Dominant	CT+TT vs CC	21 (15.3)	7 (6.7)	0.39 (0.16–0.97)	0.042
	Recessive	TT vs CT+CC	2 (1.5)	1 (1.0)	2.66 (1.02–6.91)	0.045
	Codominant	TT vs CT+CC	2 (1.5)	1 (1.0)	1.54 (0.14–17.22)	0.725
rs2740574	Additive	C allele	18 (6.6)	6 (2.9)	Reference	—
		T allele	256 (93.4)	204 (97.1)	0.42 (0.16–1.07)	0.069
	Genotypic	CC	4 (2.9)	99 (93.3)	Reference	—
		CT	10 (7.3)	6 (5.7)	0.02 (0.01–0.10)	<0.001
		TT	123 (89.8)	1 (1.0)	0.0003 (0.000–0.003)	<0.001
	Dominant	CT+TT vs CC	133 (97.1)	6 (5.7)	0.001 (0.000–0.006)	<0.001
	Recessive	TT vs CT+CC	123 (89.8)	1 (1.0)	1.57 (0.52–4.75)	0.420
	Codominant	TT vs CT+CC	123 (89.8)	1 (1.0)	913.71 (118.15–7065.95)	<0.001

Data are presented as frequency (percentage). Binary logistic regression was used to assess associations between *CYP3A4* polymorphisms and tacrolimus metabolism in relation to the risk of graft rejection. A p value <0.05 was considered statistically significant. OR, odds ratio; CI, confidence interval; em dash, reference category

p=0.027). For rs2740574, genotype frequencies among recipients were 2.9% for CC, 7.3% for CT, and 89.8% for TT. Genotype distributions differed significantly between recipients and controls. The CT genotype was significantly associated with tacrolimus dose variability (OR=0.024, 95% CI: 0.005–0.100; p<0.001). Although the T allele showed a trend toward a lower risk of graft rejection, the association was not statistically significant (OR=0.418, 95% CI: 0.163–1.073; p=0.069).

Among the *CYP3A5* polymorphisms, rs776746 was not significantly associated with tacrolimus dose requirements or graft rejection. Genotype frequencies among recipients were 81.8% for GG, 17.5% for GC, and 0.7% for CC. Neither the GC genotype (OR=1.111, 95% CI: 0.575–2.144; p=0.753) nor the CC genotype (OR=1.333, 95% CI: 0.082–21.62; p=0.839) was significantly associated with tacrolimus

Table 3 Allelic and Genotypic Frequencies of *CYP3A5* Polymorphisms in Kidney Transplant Recipients and Controls

SNP	Model	Allele/ Genotype	Kidney Transplant Recipients n (%)	Controls n (%)	OR (95% CI)	p value
rs776746	Additive	G allele	250 (91.2)	190 (90.5)	Reference	—
		C allele	24 (8.8)	20 (9.5)	0.91 (0.49–1.70)	0.771
	Genotypic	GG	112 (81.8)	84 (80.0)	Reference	—
		GC	24 (17.5)	20 (19.0)	1.11 (0.58–2.14)	0.753
		CC	1 (0.7)	1 (1.0)	1.33 (0.08–21.62)	0.839
	Dominant	GC+CC vs GG	25 (18.2)	21 (20.0)	1.12 (0.59–2.14)	0.731
	Recessive	CC vs GC+GG	1 (0.7)	1 (1.0)	0.90 (0.47–1.74)	0.759
	Codominant	CC vs GC+GG	1 (0.7)	1 (1.0)	0.68 (0.04–10.92)	0.782
rs10264272	Allelic	C allele	232 (84.7)	202 (95.3)	Reference	—
		T allele	42 (15.3)	10 (4.7)	3.66 (1.79–7.48)	<0.001
	Genotypic	TT	15 (10.9)	2 (1.9)	Reference	—
		CT	12 (8.8)	6 (5.7)	3.75 (0.64–22.04)	0.143
		CC	110 (80.3)	98 (92.5)	6.68 (1.49–29.95)	0.013
	Dominant	CT+TT vs CC	27 (19.7)	8 (7.5)	0.37 (0.17–0.83)	0.016
	Recessive	TT vs CT+CC	15 (10.9)	2 (1.9)	1.60 (0.58–4.41)	0.363
	Codominant	TT vs CT+CC	15 (10.9)	2 (1.9)	6.33 (1.41–28.34)	0.015
rs413003343	Allelic	A allele	214 (78.1)	193 (91.9)	Reference	—
		T allele	60 (21.9)	17 (8.1)	3.18 (1.80–5.64)	<0.001
	Genotypic	AA	93 (67.9)	94 (89.5)	Reference	—
		AT	28 (20.4)	5 (4.8)	0.18 (0.07–0.48)	<0.001
		TT	16 (11.7)	6 (5.7)	0.37 (0.14–0.99)	0.047
	Dominant	AT+TT vs AA	44 (32.1)	10 (9.5)	0.22 (0.11–0.47)	<0.001
	Recessive	TT vs AT+AA	16 (11.7)	6 (5.7)	5.14 (1.91–13.82)	0.001
	Codominant	AT vs AA+TT	28 (20.4)	5 (4.8)	2.18 (0.82–5.79)	0.116

Data are presented as frequency (percentage). Binary logistic regression was used to assess associations between *CYP3A5* polymorphisms and tacrolimus metabolism in relation to the risk of graft rejection. A p value <0.05 was considered statistically significant. OR, odds ratio; CI, confidence interval; em dash, reference category

metabolism. In contrast, rs10264272 was significantly associated with tacrolimus dose variability and graft rejection. Genotype frequencies among recipients were 10.9% for TT, 8.8% for CT, and 80.3% for CC. The CC genotype was significantly associated with tacrolimus dose requirements (OR=6.681, 95% CI: 1.490–29.95; p=0.013). In the allelic analysis, the T allele was significantly associated with an increased risk of acute graft rejection (OR=3.656, 95% CI: 1.789–7.475; p<0.001). For rs413003343, genotype frequencies were 67.9% for AA, 20.4% for AT, and 11.7% for TT. Compared with the AA genotype, both the AT and TT genotypes were significantly associated with tacrolimus dose variability (AT: OR=0.176, 95% CI: 0.065–0.477; p<0.001; TT: OR=0.371, 95% CI: 0.139–0.989; p=0.047). The T allele was significantly associated with increased susceptibility to acute graft rejection (OR=3.183, 95% CI: 1.795–5.643; p<0.001).

Dominant, recessive, and codominant genetic models were further analyzed to assess associations between *CYP3A4* and *CYP3A5* polymorphisms and the risk of acute graft rejection. For *CYP3A4* rs35599367, the codominant model showed a significant

association with graft rejection (OR=26.967, 95% CI: 3.615–201.144; p=0.001). For rs4646437, the recessive model was significantly associated with acute graft rejection (OR=2.656, 95% CI: 1.021–6.910; p=0.045). For rs2740574, an extremely large odds ratio was observed in the codominant model (OR=913.714, 95% CI: 118.154–7065.948; p<0.001). This result should be interpreted cautiously because sparse subgroup frequencies and small sample sizes may have inflated the effect estimate. Among *CYP3A5* variants, rs10264272 was significantly associated with graft rejection under the codominant model (OR=6.332, 95% CI: 1.414–28.337; p=0.015). Similarly, rs413003343 was significantly associated with graft rejection under the recessive model, in which the TT genotype was associated with an increased risk of acute rejection (OR=5.137, 95% CI: 1.909–13.820; p=0.001). No significant associations were identified for rs776746 under the dominant, recessive, or codominant models.

Discussion

Tacrolimus remains a cornerstone of immunosuppressive therapy in kidney transplant recipients. However, substantial interindividual variability in tacrolimus metabolism complicates the achievement of optimal therapeutic concentrations. Genetic polymorphisms in cytochrome P450 enzymes, particularly *CYP3A4* and CYP3A5, influence tacrolimus pharmacokinetics and clinical response. This study evaluated the associations of *CYP3A4* and *CYP3A5* polymorphisms with tacrolimus dose requirements and post-transplant outcomes among Pakistani kidney transplant recipients.[16] Among the *CYP3A4* polymorphisms, rs35599367 (*CYP3A4* *22) was significantly associated with acute graft rejection. The C allele was associated with an increased risk of graft rejection (OR=4.060, 95% CI: 2.455–6.715; p<0.001). Consistent with previous studies, the reduced *CYP3A4* activity associated with this variant may alter tacrolimus metabolism and increase variability in drug exposure. The heterozygous GC genotype was also significantly associated with tacrolimus dose variability. Although comparable evidence from Pakistan is limited, these findings support the importance of *CYP3A4* polymorphisms in determining tacrolimus response.[17] Similarly, rs4646437 was associated with tacrolimus dose requirements and graft rejection risk. The T allele was associated with increased susceptibility to acute graft rejection (OR=2.644, 95% CI: 1.112–6.287; p=0.027).[18] This association may reflect altered *CYP3A4* expression and tacrolimus clearance. However, because the T allele was relatively uncommon in this population, its clinical relevance should be interpreted cautiously. Previous studies have likewise reported associations between *CYP3A4* variants and altered tacrolimus metabolism. In contrast, rs2740574 (*CYP3A4* *1B) was not significantly associated with graft rejection risk.[19] Although the T allele showed a trend toward a lower risk of rejection (OR=0.418, 95% CI: 0.163–1.073; p=0.069), this nonsignificant finding should not be interpreted as evidence of a protective effect. Several analyses produced extremely large odds ratios and wide confidence intervals, particularly for rare genotype categories. These estimates were likely affected by sparse subgroup frequencies and the small number of participants carrying uncommon alleles. Their magnitude should therefore be interpreted cautiously and confirmed in larger multicenter studies with adequate statistical power.

The extremely large odds ratios observed in some genetic models were likely attributable to sparse subgroup frequencies and small sample sizes, particularly for rare genotypes, resulting in unstable effect estimates. These findings should therefore be interpreted cautiously and validated in larger cohorts. Among the *CYP3A5* polymorphisms, rs776746 (CYP3A5*3) was not significantly associated with tacrolimus dose requirements or acute graft rejection. Although this variant is known to alter enzyme activity, its clinical effect may differ among ethnic populations, as suggested by regional studies. In contrast, rs10264272 was strongly associated with tacrolimus metabolism and graft rejection risk.[20,21] The T allele was associated with an increased risk of acute graft rejection (OR=3.656, 95% CI: 1.789–7.475; p<0.001), whereas the CC genotype was associated with tacrolimus dose variability. These findings suggest that rs10264272 may influence tacrolimus pharmacokinetics and immunosuppressive response in Pakistani kidney transplant recipients. Similarly, rs413003343 was associated with tacrolimus dose requirements and susceptibility to graft rejection.[22] The T allele was associated with an increased risk of graft rejection (OR=3.183, 95% CI: 1.795–5.643; p<0.001), suggesting that this polymorphism may modulate CYP3A5 activity and tacrolimus metabolism. Several

confounding factors may have influenced the observed associations, including body weight, hepatic function, and concomitant medications that affect CYP3A activity.[23,24] Although these variables were considered in the analysis, residual confounding cannot be excluded. Future studies should include larger multicenter cohorts and comprehensive multivariable adjustment to clarify the independent contributions of *CYP3A4* and *CYP3A5* polymorphisms to tacrolimus pharmacokinetics and graft outcomes.[25]

This study has several limitations. First, it was conducted at only two transplant centers in Lahore, which may limit the generalizability of the findings to the broader Pakistani population. Second, the relatively small sample and low frequency of several genotypes may have reduced the stability and precision of the effect estimates. Third, non-probability purposive sampling may have introduced selection bias. Fourth, not all rejection episodes were confirmed by biopsy, creating a risk of misclassification. Finally, the 12-month follow-up period precluded assessment of long-term graft survival.

In conclusion, *CYP3A4* and *CYP3A5* polymorphisms were significantly associated with tacrolimus dose variability and graft outcomes among Pakistani kidney transplant recipients. *CYP3A5* variants rs10264272 and rs413003343 and *CYP3A4* variants rs35599367 and rs4646437 were associated with altered dose requirements or graft outcomes. These findings indicate a potential role for *CYP3A4* and *CYP3A5* genotyping in tacrolimus optimization; however, larger multicenter studies are needed before genotype-guided dosing can be routinely implemented in clinical practice.

References

1. Asghar RS, Saleem J, Javaid U, Zahoor M, Aziz F. Ten-year review of renal transplants in Punjab Province, Pakistan. East Mediterr Health J. 2025;31(3):191–7. doi:10.26719/2025.31.3.191
2. Knechtle SJ, Marson LP, editors. Kidney transplantation: principles and practice. 9th ed. Philadelphia, PA: Elsevier; 2024.
3. Lloberas N, Vidal-Alabró A, Colom H. Customizing tacrolimus dosing in kidney transplantation: focus on pharmacogenetics. Ther Drug Monit. 2025;47(1):141–51. doi:10.1097/FTD.0000000000001289
4. Zhao YC, Sun ZH, Li JK, Liu HY, Zhang BK, Xie XB, et al. Individualized dosing parameters for tacrolimus in the presence of voriconazole: a real-world PopPK study. Front Pharmacol. 2024;15:1439232. doi:10.3389/fphar.2024.1439232
5. Xajil-Ramos LY, Gándara-Mireles JA, Vargas Rosales RJ, Sánchez García OK, Ruano Toledo AM, Aldana de la Cruz AK, et al. Impact of CYP3A51 and CYP3A53 single nucleotide variants on tacrolimus pharmacokinetics and graft rejection risk in pediatric kidney transplant patients. Front Pharmacol. 2025;16:1592134. doi:10.3389/fphar.2025.1592134
6. Wanas H, Kamel MH, William EA, Fayad T, Abdelfattah ME, Elbadawy HM, et al. The impact of CYP3A4 and CYP3A5 genetic variations on tacrolimus treatment of living-donor Egyptian kidney transplanted patients. J Clin Lab Anal. 2023;37(19–20):e24969. doi:10.1002/jcla.24969
7. Raj TY, Fernando ME, Sujit S, Valavan KT, Harshavardhan TS, Ramanathan A. Efficacy and outcomes of CYP3A5 genotype-based tacrolimus dosing compared to conventional body weight-based dosing in living donor kidney transplant recipients. Indian J Nephrol. 2022;32(3):240–6. doi:10.4103/ijn.IJN_278_20
8. Tanaka R, Suzuki Y, Watanabe H, Fujioka T, Hirata K, Shin T, et al. Association of CYP3A5 polymorphisms and parathyroid hormone with blood level of tacrolimus in patients with end-stage renal disease. Clin Transl Sci. 2021;14(5):2034–42. doi:10.1111/cts.13065
9. Oetting WS, Wu B, Schladt DP, Guan W, Remmel RP, Dorr C. Attempted validation of 44 reported SNPs associated with tacrolimus troughs in a cohort of kidney allograft recipients. Pharmacogenomics. 2018;19(1):175–84. doi:10.2217/pgs-2017–0187
10. Balwani M. Clinical textbook of kidney transplant immunology. Chennai, India: Notion Press; 2025.
11. Tamashiro EY, Felipe CR, Genvigir FD, Rodrigues AC, Campos AB, Hirata RD, et al. Influence of CYP3A4 and CYP3A5 polymorphisms on tacrolimus and sirolimus exposure in stable kidney transplant recipients. Drug Metab Pers Ther. 2017;32(2):89–95. doi:10.1515/dmpt-2016–0036
12. Rotarescu CA, Maruntelu I, Rotarescu I, Constantinescu AE, Constantinescu I. Single nucleotide polymorphisms of

CYP3A4 and CYP3A5 in Romanian kidney transplant recipients: effect on tacrolimus pharmacokinetics in a single-center experience. J Clin Med. 2024;13(7):1968. doi:10.3390/jcm13071968
13. Hannachi I, Chadli Z, Kerkeni E, Kolsi A, Hammouda M, Chaabane A, et al. Influence of CYP3A polymorphisms on tacrolimus pharmacokinetics in kidney transplant recipients. Pharmacogenomics J. 2021;21(1):69–77. doi:10.1038/s41397–020–00179–4
14. Xuan NT, Hop VQ, Kien TQ, Toan PQ, Thang LV, Binh HT, et al. Frequencies and association of CYP3A5 polymorphism with tacrolimus concentration among renal transplant recipients in Vietnam. Transplant Proc. 2022;54(8):2140–6. doi:10.1016/j.transproceed.2022.07.009
15. Nissenson AR, Fine RN, Mehrotra R, Zaritsky J, editors. Handbook of dialysis therapy e-book. 6th ed. Philadelphia: Elsevier Health Sciences; 2022.
16. Alatorre-Moreno EV, Saldaña-Cruz AM, Pérez-Guerrero EE, Morán-Moguel MC, Contreras-Haro B, López-de La Mora DA, et al. Association of CYP3A4-392A/G, CYP3A5-6986A/G, and ABCB1-3435C/T polymorphisms with tacrolimus dose, serum concentration, and biochemical parameters in Mexican patients with kidney transplant. Genes (Basel). 2024;15(4):497. doi:10.3390/genes15040497
17. Khan AR, Raza A, Firasat S, Abid A. CYP3A5 gene polymorphisms and their impact on dosage and trough concentration of tacrolimus among kidney transplant patients: a systematic review and meta-analysis. Pharmacogenomics J. 2020;20(4):553–62. doi:10.1038/s41397–019–0144–7
18. Colvin RB, Chang A, Cornell LD. Diagnostic pathology: kidney diseases e-book. 3rd ed. Philadelphia: Elsevier Health Sciences; 2023.
19. Chen L, Prasad GR. *CYP3A5* polymorphisms in renal transplant recipients: influence on tacrolimus treatment. Pharmacogenomics Pers Med. 2018;11:23–33. doi:10.2147/PGPM.S107710
20. Zuo XC, Ng CM, Barrett JS, Luo AJ, Zhang BK, Deng CH, et al. Effects of CYP3A4 and CYP3A5 polymorphisms on tacrolimus pharmacokinetics in Chinese adult renal transplant recipients: a population pharmacokinetic analysis. Pharmacogenet Genomics. 2013;23(5):251–61. doi:10.1097/FPC.0b013e32835fcbb6
21. Hariharan S, editor. Long-term care of kidney transplant patients. Oxford University Press; 2024.
22. Wanas H, Kamel MH, William EA, Fayad T, Abdelfattah ME, Elbadawy HM, et al. The impact of CYP3A4 and CYP3A5 genetic variations on tacrolimus treatment of living-donor Egyptian kidney transplanted patients. J Clin Lab Anal. 2023;37:e24969. doi:10.1002/jcla.24969
23. Charfi R, Bacha MM, Ben Fadhal M, Ferchichi K, El Jebari H, Gaies E, et al. The effects of the CYP3A5*3 variant on tacrolimus pharmacokinetics and outcomes in Tunisian kidney transplant recipients. Tunis Med. 2023;101(10):738–44.
24. Alam A, Amanullah F, Baig-Ansari N, Lotia-Farrukh I, Khan FS. Prevalence and risk factors of kidney disease in urban Karachi: baseline findings from a community cohort study. BMC Res Notes. 2014;7(1):179. doi:10.1186/1756–0500–7–179
25. Khan AR, Raza A, Firasat S, Abid A. CYP3A5 gene polymorphisms and their impact on dosage and trough concentration of tacrolimus among kidney transplant patients: a systematic review and meta-analysis. The Pharmacogenomics Journal. 2020;20(4):553–62.